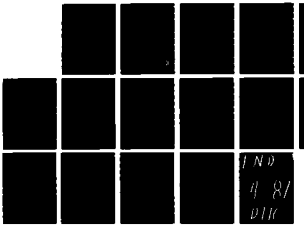
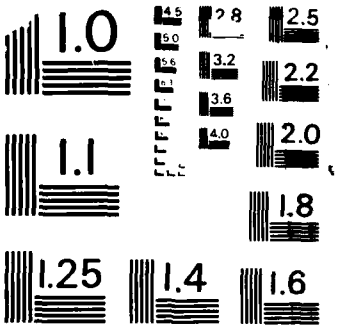
AD-A177 831 DEVELOPMENT OF CENTRAL NERVOUS SYSTEM RADIOPROTECTORS (U) CHILDREN'S HOSPITAL OF PHILADELPHIA PA JOSEPH STOKES RESEARCH CENTER J M YUHAS MAY 82 1/1

UNCLASSIFIED DAMD17-81-C-1082 F/G 6/15 NL





AD_____

"Development of Central Nervous System Radioprotectors"

Annual Report

John M. Yuhas, Ph.D.

May, 1982

Supported by

U.S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract DAMD17-81-C-1082

Joseph Stokes Res. Center
Children's Hospital of Philadelphia
Philadelphia, PA 19104

The findings in this report are not to be construed as an offical Department of the Army position unless so designated by other authorized documents.

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO. ADA177831 / 3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) DEVELOPMENT OF CENTRAL NERVOUS SYSTEM RADIOPROTECTORS	5. TYPE OF REPORT & PERIOD COVERED Annual (5/1/82-4/30/82) / 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) John M. Yuhas, Ph.D.	8. CONTRACT OR GRANT NUMBER(s) DAMD17-81-C-1082
9. PERFORMING ORGANIZATION NAME AND ADDRESS Joseph Stokes Research Center, Childrens Hospital of Philadelphia, Philadelphia, PA 19104	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62734A.3M162734A875.AK.092
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Res. & Develop. Command, Fort Detrick, Frederick Maryland 21701	12. REPORT DATE May, 1982 / 13. NUMBER OF PAGES 30
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report) Unclassified / 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

radioprotection; central nervous system

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This project is attempting to develop practical drugs which would be able to protect the central nervous system from acute functional injury produced by ionizing radiation. By practical is meant drugs which could be self administered and which do not, by themselves, alter the functional capacity of the central nervous system. Assays are being developed which allow quantitation of functional injury and preliminary identification of appropriate candidate drugs is underway.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

SUMMARY

During the past funding period we have developed two assays for CNS radiation injury, identified the reason why most radioprotective drugs do not protect the CNS and developed a simple chemical assay which will help us to identify those radioprotective drugs which would protect the CNS, even when the candidate drugs are only available in minute quantities and of unknown purity. The fact that drugs which would protect the CNS are exactly the reverse of those which might be effective in cancer therapy should dispel any appearances of conflict of interest and will benefit the CNS protector development because highly effective drugs which fail in therapy are likely to be effective in the CNS.

OBJECTIVE

The overall objective of this research proposal is to identify and develop a drug which would protect military personnel from the acute functional incapacitation which accompanied ionizing radiation exposure of the central nervous system (CNS). Implicit in this objective is the requirement that this drug be practical for such an application, i.e., it must be amenable to self-administration, possess no debilitating side-effects of its own and have a prolonged duration of action.

APPROACH

Many radioprotective drugs are known, but none of those presently available is able to offer significant protection to the CNS, unless one includes those agents which do so via the induction of systemic physiologic excursions (Yuhas and Storer, 1969), which are themselves functionally debilitating. As a logical approach to this problem we proposed to identify the basis for poor CNS protection, (by even the most active drugs presently available), to identify the chemical basis for this characteristic in the hope of predicting which drugs might protect the CNS, and to then test these appropriate drugs in an assay system which would be predictive of the protection which the drug would offer against radiation induced functional incapacitation in man. Implicit in this design would be the development of a relevant assay for CNS radiation injury, since none were available in mice/rats and those used in larger species could not be adapted. From the stand point of time, expense, logical progress and the likelihood of success, this approach appeared preferable to empirical screening methods.

In describing our progress and our proposed further studies, each area of investigation will be treated separately in order to avoid confusion and to point out the logical inter-relationships of the individual areas.

PROGRESS REPORT

Central to the successful completion of this project would be the availability of an assay system which would allow us to identify those agents which would be practical CNS radioprotectors in a military situation. Most of the radiobiologic assays which have been developed for CNS injury either concentrate on the late consequences of radiotherapeutic exposures, or involve large mammals which would not lend themselves to the scale of investigation required by the present proposal. This is not to say that monkeys or other large mammals might not eventually be needed to scale up and verify the activity of drugs we identify, but their use would be inappropriate at this preliminary stage. We proposed to investigate a series of different assays and we describe below our progress thusfar.

CNS Injury Assays - As the precision and accuracy of an assay increases, its relevance and similarity to the in vivo situation declines. Sooner than focus on one end of the spectrum or the other, we initially proposed to study three types of assays, and to then select the most appropriate one or ones for our analyses of CNS radioprotection.

The three types of assays chosen were: morphologic, functional and lethal. The morphologic assays were designed to measure the amount of radiolabelled drug which escaped the circulation and crossed the blood brain barrier in control animals and in animals given graded doses of CNS radiation between 0 and 24

hours before. During the course of these studies, a typical one being given in Table I, we employed the Fisher 344 rat, radiation doses of 0 to 50,000 rads, and three agents which do not normally pass the blood-brain barrier in significant quantities: ^{35}S-sodium thiosufate, ^{125}I-bovine serum albumin, and ^{14}C-WR-2721. This latter agent was used as an indicator post-irradiation, not as a pre-irradiation protector in these studies (cf. below regarding the limited permeability of WR-2721 across the blood brain barrier). As typified by the experiment given in Table I, we could demonstrate radiation enhancement of passage of these agents across the blood brain barrier, but the effect did not appear dose responsive, thereby preventing us from using it, as such, as a means of estimating levels of radioprotection. It remains possible that appropriate modification of experimental conditions, might allow us to construct dose response curves from such data, but the inherent variability of these systems coupled with the large amounts of time and animals involved would not appear to make this our most promising approach. We will propose below limited further studies in the hope of developing this into a simple assay system.

The functional assays we have studied thusfar include the following: the ability of control and irradiated mice to negotiate a rotating rod for a food reward, the sensitivity of the same two groups to anesthetics, and the ability of the same two groups to perform a task they had been trained to do prior to irradiation.

This rotarod assay is a standard method for assessing drug induced peripheral neurotoxicity, and we hoped that it would also detect radiation injury to the brain. The methodology was taught to us by Dr. Richard Johnson, Roswell Park Memorial Institute, and was employed by us in preliminary assays for CNS radiation injury. To summarize succinctly, this assay method did not detect the injury induced by CNS doses as high as 10,000 rads within the first

24 hours, and was fraught with high variability. Accordingly, this assay was abandoned.

The two other functional assays proved to be more promising. Table II summarizes the results of our anesthetic assays in which the rats are anesthetized with ketamine at the time of exposure and assayed for sensitivity to anesthesia induced by sodium pentobarbital either 1 or 24 hours later. We record the following parameters: duration of ketamine induced sleep (radiation delivered 2-3 minutes after injection), time between injection of sodium pentobarbital and sleep, and duration of sodium pentobarbital induced sleep. As shown in Table II, at both 1 and 24 hours after irradiation the two measures of sodium pentobarbital sensitivity demonstrate dose responsive CNS radiation injury, and radiation induced prolongation of ketamine induced sleep is apparent. The effect at 24 hours is smaller than that observed at 1 hour, but remains the better assay for our purposes, since one can avoid the complications of administering the sodium pentobarbital at short intervals after the radioprotective drug, such as would be required if the assay were conducted at 1 hour after exposure.

The third functional assay came to us as a spinoff of other studies being conducted by Dr. Giulio D'Angio and Dr. Len Bruno, of the Departments of Radiation Therapy and Neurosurgery of the University of Pennsylvania and the Children's Hospital of Philadelphia. These investigators were attempting to model the late radiation injury which is observed in children who receive CNS radiation therapy. Their model calls for the administration of various treatments to the brains of 17 day old rats and then analysis of their ability to learn a lever pressing/reward system. The endpoint in their studies is the amount of time it takes for a rat to achieve a 90% correct rate of response as a function of the treatment received some 2 months or more ago. The control rats,

who learned this task rapidly, were normally being discarded and we had them save these rats for us such that we could use them to determine whether radiation exposure would comprise the ability of the rats to perform a task they had already mastered. In the first experiment, a total of 16 rats were randomly assigned to CNS dose levels of 0 (n=5), 5000 (n=6) or 10,000 (n=5) rads, and were tested 24 hours after exposure. Testing immediately before exposure, revealed that they had retained their greater than 90% correct response rate to the light signal which gave them a sweetened milk reward when the correct lever was pressed. These rats were then irradiated and re-tested 24 hours later.

Figure I summarizes the results of these preliminary studies and demonstrates that radiation induces two effects. First the rate at which the rats respond to the light cue, correctly or incorrectly, declines with increasing dose, in spite of the fact that these rats had been deprived of food overnight. Second, and perhaps more specifically, the correct response rate drops from 92% at 0 rads, to 78% at 5000 rads, to 56% at 10,000 rads. Therefore, radiation has reduced not only the motivation to try to get the reward, but also the previously learned manner of producing a correct response*. These studies have been successfully repeated three times.

---

* Dr. Peter Block, of our physics section, has determined that a rat receiving 10,000 rads to the CNS receives an average of 300 rads to the whole body (which is protected by a cerrobend collimator and shield, making it unlikely that the results observed are the product of non-specific whole body effects. A simple experiment will test this directly in the coming year.

The last type of assay system we have studied is an adaptation of the repeated dose to death assay system we previously reported for the mouse (Yuhas, 1968). Rats are given doses of 10,000 rads at 10 minute intervals until death. From our accumulated studies, it has been observed that Fisher 344 rats tolerate an average of 14.1 ± 0.56 doses before death, and an average of 8.9 ± 0.40 doses before losing their righting reflex. These estimates correlate quite closely with those previously reported for the mouse (Yuhas, 1968). In addition, we have confirmed, in the rat, that high doses of WR-2721 reduce tolerance to these repeated doses (data not shown) and that lower doses, while avoiding this combined toxicity, fail to give evidence of CNS protection (Yuhas and Storer, 1969). Similar results have been obtained with two analogues of WR-2721, WR-3689, and WR-44923, and the failure of these agents to protect the CNS has been resolved through our studies of drug characteristics described below.

Our choices at present for assaying CNS radiation injury would include radiation induced alterations in the motivation and ability to perform a learned task and the radiation doses to incapacitation and death. During the coming year we propose to continue refinement of these assay systems as described below.

Basis of Poor CNS Protection - As pointed out above, the general rule is that standard radioprotective drugs do not offer significant protection of the CNS (Yuhas and Storer, 1969). We observed no protection of the mouse against radiation induced CNS lethality when the WR-2721 was injected shortly before irradiation (Yuhas and Storer, 1969) or of the rat against late spinal cord injury (Yuhas, 1979). In contrast to these results Jacobus et al. (D. Davidson, Personal Communication) reported that WR-2721 could protect the monkey against radiation induced early transient incapacitation. During the coming year we plan to resolve this discrepancy by determining whether WR-2721 can protect the

rat against radiation induced loss of motivation and ability to perform a previously learned task, i.e., we know that it is possible to protect rodents against CNS radiation injury, (Yuhas and Storer, 1969) using physiologic excursions and the small level of protection seen by Jacobus may be the product of the endpoint studied.

Whatever the resolution of this question, it is clear that standard radioprotective drugs either offer no CNS protection or such low levels of protection that practical exploitation is impossible, in spite of the fact that they can increase the resistance of a variety of other normal tissues by factors of 2.5 - 3.0 (Yuhas et al., 1980). It appeared to us, therefore, that the most efficient way to develop an effective CNS protector would be to understand why WR-2721 and other standard protective drugs did not protect the CNS. As outlined in our initial proposal, the proximate reason why WR-2721 did not protect the CNS could be traced to the very limited amounts of the drug which entered the brain following injection (Yuhas, 1980). Two factors contributed to this poor absorption: restricted entrance of the drug through the blood brain barrier and a short serum half-life.

The question addressed during the past 9 months has been why WR-2721 is so effectively restricted and what chemical characteristic of the drug is responsible for this restriction. Classical pharmacology would suggest that the characteristic most likely to restrict the ability of drugs of this type to cross the blood-brain barrier would be the relative solubility of the drug in lipid and water. This is normally expressed as the octanol: water partition coefficeint which is determined by standard laboratory methods. WR-2721 proved to be very hydrophilic with an octanol: water partition coefficient of 0.0004, i.e., given equal access to octanol and water, only 0.0004 parts would be soluble in octanol for every 1 part which solubilized in water. This factor

alone would appear able to account for the limited CNS absorption, but sooner than leave this as an isolated observation, we wished to pursue this in greater detail, such that we could determine how this drug might be modified or replaced such that CNS absorption could be obtained.

Table III summarizes the octanol: water partition coefficients which were observed for WR-2721, its symetrical disulfide and its free sulfhydryl. Both of the derivatives are less hydrophilic than WR-2721 and cross barriers which restrict hydrophilic drugs more readily. Due to the short half-life of all of these drugs in vivo, it is difficult to show that the two dephosphorylated derivatives of WR-2721 achieve significant levels in the CNS, but the fact that they can readily cross normally restrictive barriers can be demonstrated in our in vitro model of the blood brain barrier, i.e., the red blood cell. Figure II is a plot of the amount of drug absorbed by red blood cells and liver cells as a function of time. Two points are apparent, the liver readily absorbs both WR-2721 and its dephosphorylated derivatives, but the red blood cell restricts the absorption of WR-2721 while allowing its dephosphorylated derivative to enter freely. The combination of these in vivo and in vitro studies would suggest that the ideal CNS protector would be far less hydrophilic than WR-2721 and possess a sufficiently long serum half life such that significant absorption by the CNS would be possible. A variety of procedures (e.g., mannitol infusion) can be used to open the blood brain barrier and thereby allow normally restricted drugs to enter the CNS, but these approaches would not appear worthy of further pursuit, except as a means of investigating specific questions. These procedures would be impractical for the overall objective of this research because they would not be amenable to self-administration and can, by themselves, produce debilitating side effects.

Given the basic argument that a CNS protector had to be less hydrophilic

than WR-2721, we were presented with the opportunity to rationally select candidate drugs for study in our CNS radiation injury assays, but were also presented with technical difficulties regarding exactly how we would determine the partition coefficient for these drugs. Briefly, these problems were as follows: many of the potential agents are available in limited quantities of unknown purity; many of these drugs do or can break down during the partitioning procedure; and highly quantitative assay systems are not available for most of the agents to be studied (e.g., we used ^{14}C-labelled drugs for our studies on WR-2721 and its derivatives, but most of the agents we wished to study are not available in radiolabelled form). In brief, it appeared that we had uncovered the basic drug characteristic which limited CNS protection, but would be unable to measure it accurately for most of the drugs. Our initial efforts concentrated on the use of a variety of quantitative techniques for measuring sulfur, an element common to all of the radioprotectants. Reasonable progress was being made in this area but this still did not overcome the problem of limited sample size and drug purity. This latter problem was particularly worrisome, since a small amount of a far less hydrophilic contaminant can grossly alter the estimated coefficients, and we had no way to estimate the amount of contaminant present.

This problem was eventually resolved when we returned to the basic principles of chromatography. The octanol: water partition coefficient of a drug is a reflection of a drugs polarity, i.e., highly polar drugs tend to be hydrophilic and vice versa. The distance which a drug migrates on a chromatogram is also a function of drug polarity, but in this case relative to the polarity of the solvent system used to develop the chromatogram. We reasoned therefore that since the molecular weight (another variable which can affect migration in chromatography) of the compounds we are interested in fell

in a narrow range of 150 -300, we might be able to use chromatographic methods to measure each drugs relative hydrophilicity. Small samples would be required and more importantly, we would be able to detect contaminants or more precisely focus our attention on the parent compound. To verify that the system worked, we selected a series of nitroimidazoles, whose octanol: water partition coefficient were known, and determined the relative distances they migrated (R_f) in varying combinations of a non-polar (carbon tetrachloride) and polar (ethanol) solvent. Figure III is a plot of the chromatographic data as a function of the respective partition coefficients for each drug, and a linear relationship between the two is apparent. The particular solvent system used in these studies was not useful for the study of radioprotectants, all of which are more hydrophilic than the nitroimidazoles, so we chose to use the combination of isopropanol and ammonium hydroxide.

Using this system, we verified that WR-2721 and its dephosphorylated derivatives yielded migration patterns which correlated with their partition coefficients (Figure IV), and verified our expectation that phosphorothioates, as a general class, are highly hydrophilic and therefore inappropriate for CNS protection (Figure V, top). Preliminary studies have also been conducted with non-phosphorothioates which are presently being studied under the joint sponsorship of the Walter Reed Army Institute of Research and the National Cancer Institute, and a fourth drug, diethyldithiocarbamate, which has been reported in the literature to be marginally effective as a protector. As shown in Figure V (bottom) these four agents are all far less hydrophilic than WR-2721, and therefore more likely to offer radioprotection to the CNS. The numbers in parentheses for each agent are the dose reduction factors or factor increases in radiation resistance which each agent offers against radiation induced bone marrow death when administered at one-half the maximum tolerated

dose (D.Q Brown, Personal Communciation). Therefore, it would appear possible to develop a highly effective radioprotector which has a far greater likelihood of penetrating the CNS. Whether even less hydrophilic drugs will be required or will offer even more effective CNS protection can only be determined via direct testing.

PROPOSED STUDIES

Our progress during the past year can be summarized as follows: we have developed at least two assay systems for the quantitation of CNS radiation injury which would appear to bear on the practical problem at hand and we have identified the reason why standard radioprotectants offer little to no radioprotection of the CNS, and have developed a simple means of identifying those compounds which are most likely to offer CNS protection. Preliminary evaluation of a candidate compound can be performed with as little as 2.5 mg, if necessary.

During the coming year, we propose to expand on these observations, further refine our systems and perform preliminary testing of agents which are more likely to protect the CNS than is WR-2721.

CNS injury Assays - As pointed out above, we have three assays for quantitating the debilitating effects of CNS irradiation: anesthetic sensitivity, performance of a previously learned task, and incapacitation and death under the influence of repeated exposures. While interesting, the anesthetic sensitivity assays will be de-emphasized since their use immediately after exposure suffers from potential interactions with the radioprotective drug which persists through one hour after exposure, and at 24 hours after exposure,

the dose response curve is less able to discriminate dose dependence (Table II). Although the radiation induced inhibition of the learned task performance is far more labor intensive, it would appear to represent the closest approximation of the practical problem being addressed. The additional time required to train and test each animal (30 minutes per day) would appear to represent a worthwhile investment when considered relative to the information to be obtained. Since the studies being conducted by Drs. D'Angio and Bruno provide us with only limited numbers of animals at erratic intervals, we are proposing to rely on their expertise and instrumentation, but to train and test the animals ourselves. Using this system, we propose the following series of experiments:

1. characterization of the complete dose response curve for radiation inhibition of the rate of task performance, and the frequency of correct "answers",
2. verification that the small total body dose received (300 rads following administration of 10,000 rads to the CNS) is not responsible for the observed effects on learned task performance,
3. characterization of the time course of the radiation induced effects on learned task performance, such that comparability with the practical problem may be determined,
4. analysis of the ability of known CNS protectors (e.g. para-aminopropiophenone or hypoxia) can protect against this radiation insult, thereby showing that this type of injury can and should be protected against by a truly effective candidate drug, and
5. assay of WR-2721 in this system to determine whether the failure of WR-2721 to protect the CNS is endpoint dependent.

The lethality assay is about as refined as it will ever be and we propose no further development of this model, but intend to retain it as one of our assays for radioprotection in order to provide a second developed system which requires minimal expenditures of time and effort.

Last, we are proposing to continue attempts to develop an assay based on radiation induced alterations in blood brain barrier permeability. Two specific approaches are proposed: non-invasive monitoring of the iothalmate concentration in the brain in control and irradiated rats relative to the concentration in their circulation; and passage of semi-restricted isotopes into the brain of control and irradiated rats.

Dr. Peter Bloch, of our Physics Division, has perfected a non-invasive X-ray detector which can be used repeatedly on living tissue to detect parts per million of such trace elements as lead and mercury in living tissue. We are presently engaged in a collaborative study designed to quantitate the rates of clearance of iothalmate from the circulation in rats via monitoring of the concentration of iodine in the blood as it passes through the tail. We propose, here, pilot studies, to determine whether the same methodology can be used to assay total vascular volume and/or residual iothalmate concentration in the brain of control and irradiated rats. It is our suspicion that part of the limitation of the tracer uptake assays we have performed in the past was the dilution phenomenon which accompanied edema in irradiated brains. By determining total drug present this problem would be avoided and the non-invasive nature of the assay would allow for repeated testing in individual animals.

In the hope of developing the blood brain barrier leakage assay for the purposes of quantitating CNS radiation injury, we will not use the almost totally restricted agents we have used in the past (sodium thiosulfate, WR-2721

and bovine serum albumin) and concentrate on agents which pass the barrier but only in limited amounts. The specific agent we are proposing to test is ^{14}C-urea, which is far less hydrophilic than the agents previously used (PC = 0.0016), but still has limited access to the brain parenchyma.

Identification of Candidate Drugs - Although it would be invalid to assume that the more effective a given drug is in protecting against radiation induced bone marrow injury the more effective it would be in protecting the CNS, the effectiveness of a given drug in protecting the bone marrow provides us with an index of how effective the drug will be if it is given free access to a tissue. For the evaluation of the inherent protective capability of a drug (= ability to protect the bone marrow) we rely on two sources: the compendium of the USAMR&DC Anti-Radiation Drug Development Program (Sweeney, 1979) and on-going studies on promising drugs. These ongoing programs include assay of the protective activity of selected drugs in a variety of normal tissue systems by D.Q. Brown (Philadelphia, PA) under NCI sponsorship, analysis of the ability of selected drugs to protect against fission neutrons, being conducted by Dr. C.P. Sigdestad (Louisville, KY) and a variety of studies which funnel through Dr. David Davidson, WRAIR, the technical representative on this contract.

These data provide us with an evaluation of the inherent potential of a drug, and we then will analyze the hydrophilicity of each drug as described above. Before entering into a definitive analysis of drugs with useable inherent protective capability, we will first design a solvent system which will allow us to study and quantitate (via the simple methods we have developed) drugs with partition coefficients as high as 0.5 - 0.7 and as low as 0.0001. At present we must use different solvent systems for each partition coefficient range, and the likelihood of developing a single two part system which covers

the entire range is unlikely. A three part system is proposed in which two of the three solvents will be as similar as possible for 4 of the 5 characteristics which govern polarity but will show sufficient variation in the 5th that the entire range or at least the vast majority of it can be studied in a continuous system.

We then propose to classify each of the most promising protective drugs in terms of its hydrophilicity, such that one can set priorities regarding the likelihood of obtaining CNS protection. We propose, at the outset, to analyze as many drug classes as possible, in the hope of being able to exclude particular classes of drugs, much as we have been able to with phosphorothioates. From those which both possess inherent protective capability and appropriate solubility characteristics, we will construct a rank ordering for priorities for further testing which should maximize our likelihood of success and minimize non-productive pursuits.

Analysis of CNS Radioprotection - Three agents have already been identified which offer significant radioprotection in the bone marrow and are far less hydrophilic than WR-2721. These drugs (Figure III) are: WR-157113, WR-2529 and NSC-62857. Their structures are given in Table IV. During the coming year we propose to test them for the ability to protect the CNS using both the learned task and lethality assay systems. The drugs will be injected at 50% and 100% of the maximum tolerated dose at 15 or 30 minutes before irradiation. If protection is observed in either of these systems, we will proceed with the sequence of studies designed to analyze the practical aspects of using such a drug. These include, verification that the protection is not the product of physiologic excursions, relationship between drug dose injected and protection observed, time course of radiation protection and activity following oral

administration. Full experimental protocols for these studies have been included in the original proposal and are not repeated to avoid repetition.

Pending the outcome of our assay development studies, we will continue to assay learned task performance at 24 hours after exposure. Depending on the outcome of these studies, this protocol will be altered to maxilmize dose discrimination and increase accuracy and repeatability.

As additional agents become available they will be assigned a priority as described above and be introduced into testing as appropriate.

Table I. Effects of central nervous system irradiation on the ability of this tissue to absorb S-35 labelled sodium thiosulfate.

Radiation Dose[a] (rads)	Counts per Minute per mg		
	Plasma[b]	Brain	Liver
0	17.2 ± 0.3	5.1 ± 0.4	11.1 ± 1.2
2500	16.1 ± 0.2	6.7 ± 0.2	12.3 ± 1.4
5000	17.4 ± 0.6	6.9 ± 0.3	10.9 ± 0.9
10000	16.7 ± 0.3	6.5 ± 0.3	12.3 ± 1.5

a - N = 5-6 rats per point; an i.v. injection of S-35 sodium thiosulfate (10^5 cpm) was given 1 hour after exposure and the animals were killed 24 hours later.

b - cpm per lambda of plasma.

Table II. Effects of CNS irradiation on the sensitivity of rats to ketamine or sodium pentobarbital.

Radiation Dose	Ketamine Sleep Time (min.)[a]	Time Post-Rad.	Pentobarbital Sens. Resp. Time[b]	Duration[c]
0	12.4 ± 0.6	1 hour[d]	5.3 ± 0.3	47.5 ± 2.3
	13.2 ± 1.1	24 hours	4.5 ± 0.4	58.5 ± 2.1
2500	21.4 ± 0.9	1 hour	2.0 ± 0.1	76.3 ± 3.5
	28.5 ± 1.2	24 hours	1.9 ± 0.4	61.6 ± 2.1
5000	34.1 ± 1.8	1 hour	2.3 ± 0.4	87.5 ± 3.7
	35.0 ± 3.5	24 hours	2.2 ± 0.2	81.0 ± 6.7
10000	30.1 ± 1.4	1 hour	2.1 ± 0.3	91.0 ± 3.0
	33.0 ± 1.4	24 hours	2.3 ± 0.5	77.5 ± 2.3

a - rats were given an injection of 35 mg/kg of ketamine and 2-3 minutes later received their CNS radiation; increases in this sleep time represent prolongation of sleep in animals who were sleeping at the time of irradiation.

b - Response time equals the time between an injection of 30 mg/kg of nembutal and the time the animals lost their righting reflex.

c - Duration of sleep measured from the time the animals lost their righting reflex (as in b) and the time they regained it.

Table III. Octanol:water partition coefficients for WR-2721 and its dephosphorylated analogues.

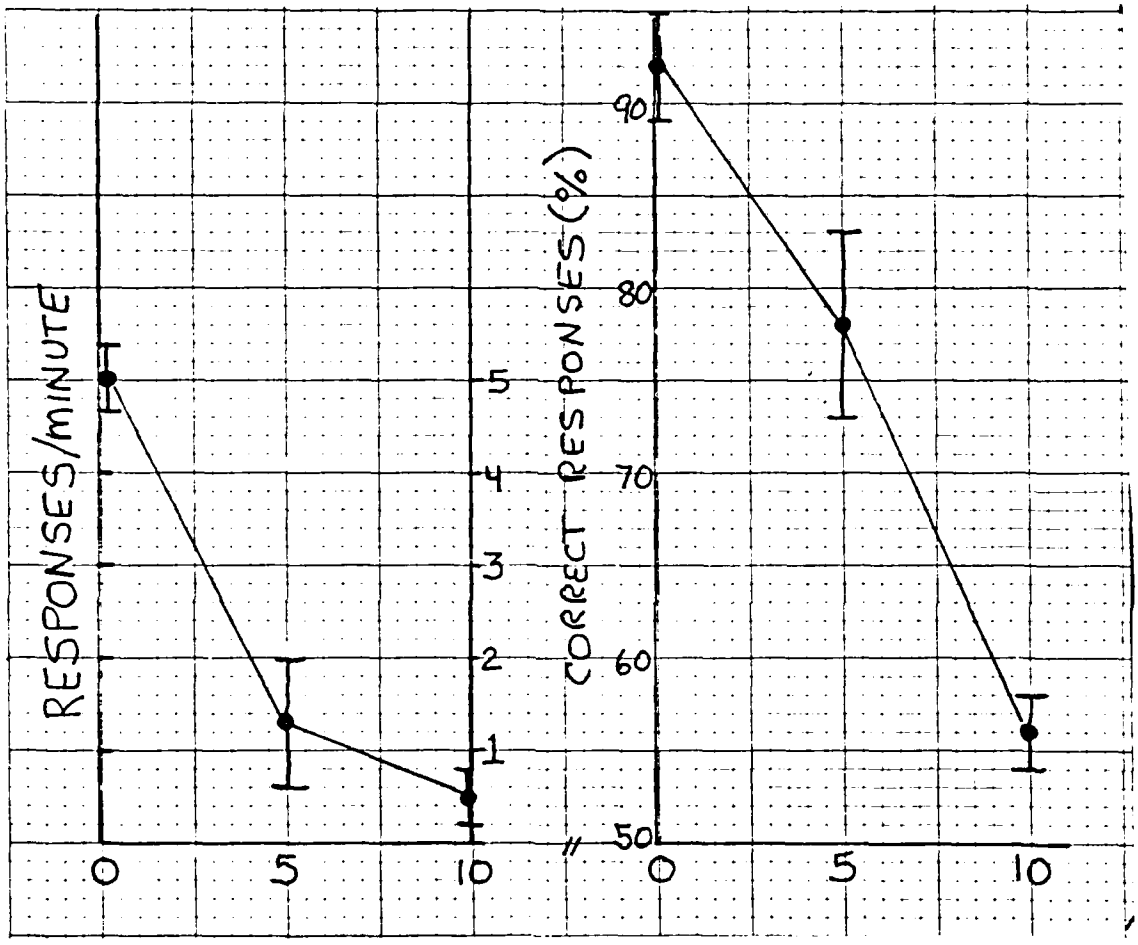
Drug	Formula	Octanol:H_2O Partition Coefficient
WR-2721	$H_2N(CH_2)_3NH(CH_2)_2SPO_3H_2$	0.0004
WR-33278	$H_2N(CH_2)_3NH(CH_2)_2S-$	0.0015
WR-1065	$H_2N(CH_2)_3NH(CH_2)_2SH$	0.0046

Table IV. Candidate Drugs for CNS radioprotection.

Drug	Formula	DRF at MTD/2[a]
NSC-62857	$H_2N(CH_2)_3SC(=NH)NH_2$	1.74 ± 0.06
WR-2529	$H_2NCO(CH_2)_2NH(CH_2)_2SH$	1.6
WR-157113	$H_2N(CH_2)_2SS_2O_3H$	1.5

a - Dose reduction factor against radiation induced hematopoietic death when one-half of the maximum tolerated dose was injected 15 minutes before irradiation (D.Q. Brown, Personal Communication).

Figure I. Effects of CNS irradiation, given 24 hours earlier, on the ability of rats to perform a learned task.



for one to two weeks prior to exposure the rats are kept without food overnight and then placed in a testing box in which a correct response to a light cue provides a sweetened milk reward; more than 95% of the animals can learn a correct response rate in excess of 90% within 4-5 days. They are then exposed and 24 hours later re-tested to determine their ability to perform this learned task.

Figure IIa. Absorption of WR-2721 in vitro by rat liver cells (circles and triangles) and by red blood cells (squares) as a function of time. Closed symbols = 37 degrees and open symbols = 4 degrees. The triangles in the liver cell studies included heparin (which was used to harvest the rbc) to control for the possible contribution of this anticoagulant to the results.

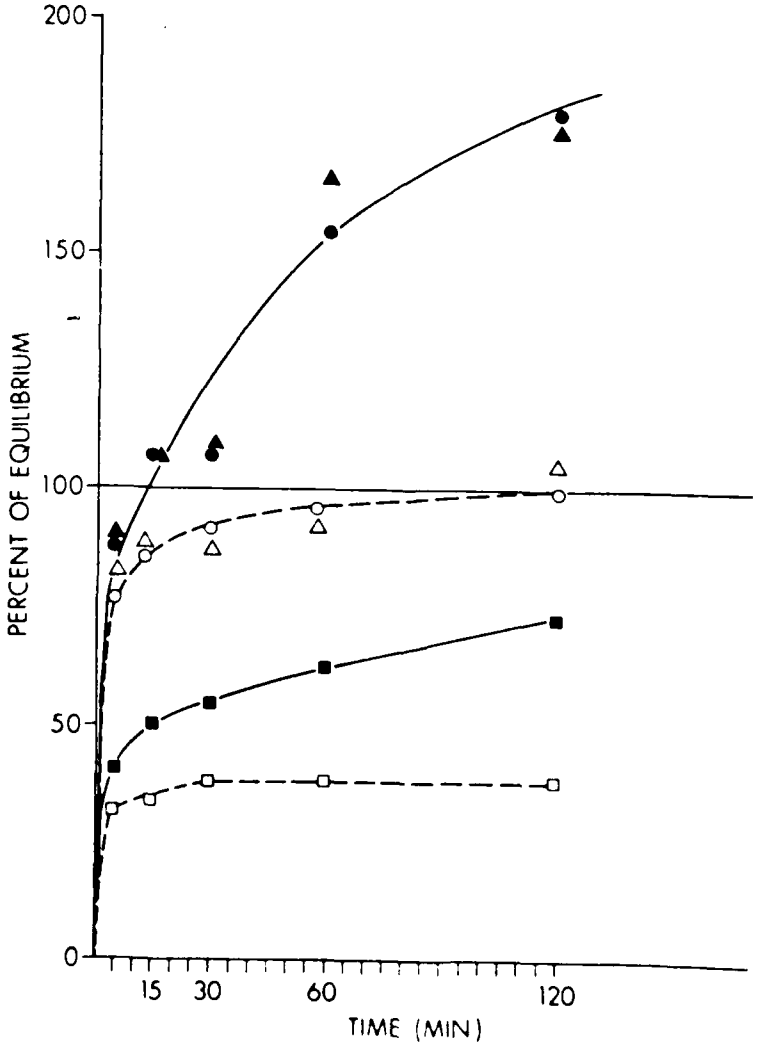


Figure IIb. Absorption of WR-2721 and a mixture of its dephosphorylated analogues by red blood cells as a function of time. RSH2PO3 = WR-2721 and RSSR/RSH = a mixture of its symetrical disulfide and its free sulfhydryl. More recent studies (data not shown) have demonstrated the the RSH is absorbed far more readily than the RSSR.

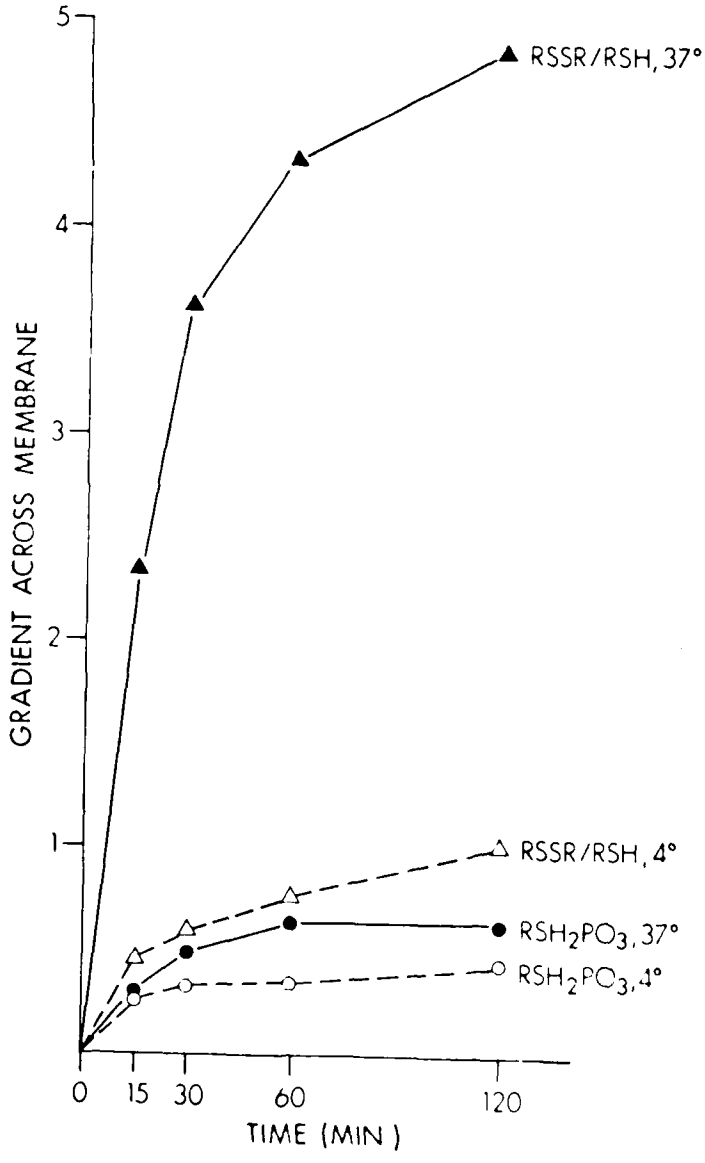


Figure III. Relative migration (R_f) of four nitroimidazoles in a carbon tetrachloride:ethanol solvent system as a function of their octanol water partition coefficients.

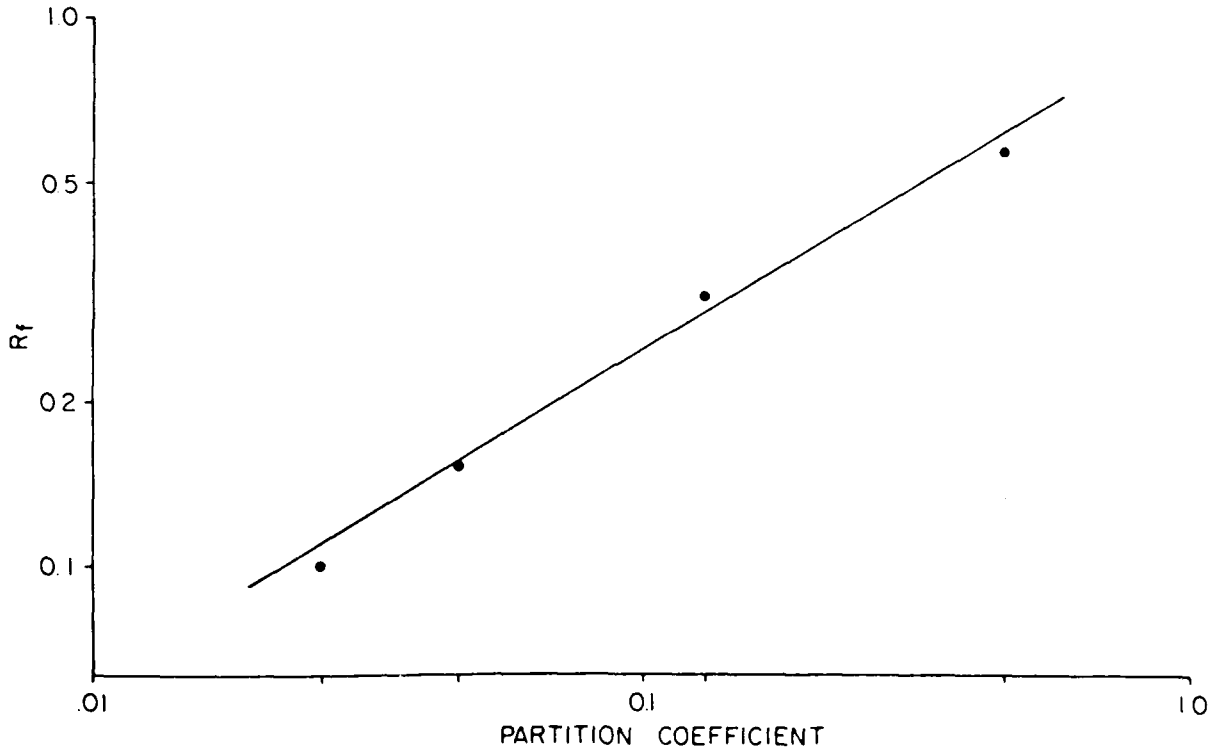


Figure IV. Relative migration (R_f) of three phosphorothioates and of their symetrical disulfides and free sulfhydryls in an isopropanol ammonium hydroxide solvent system. (— —) = WR-2721; (····) = WR-44923; and (——) = WR-1638.

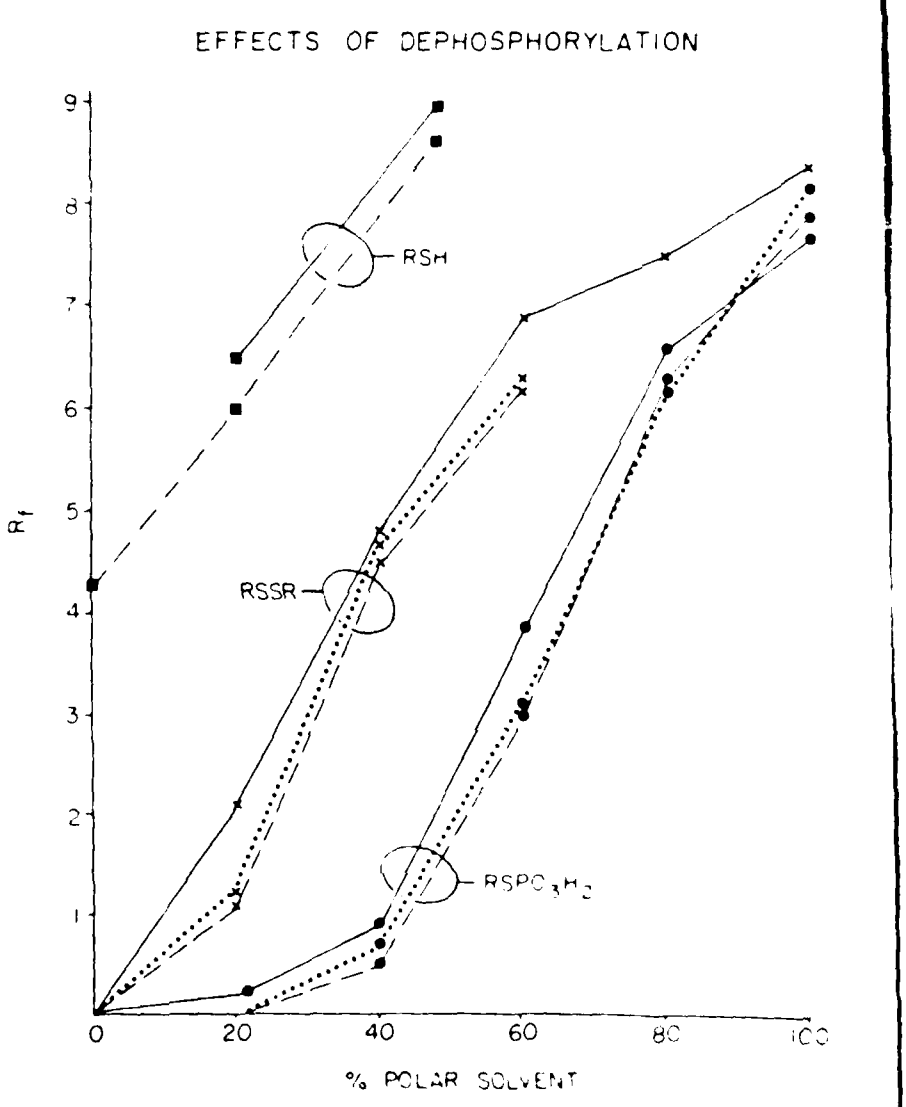
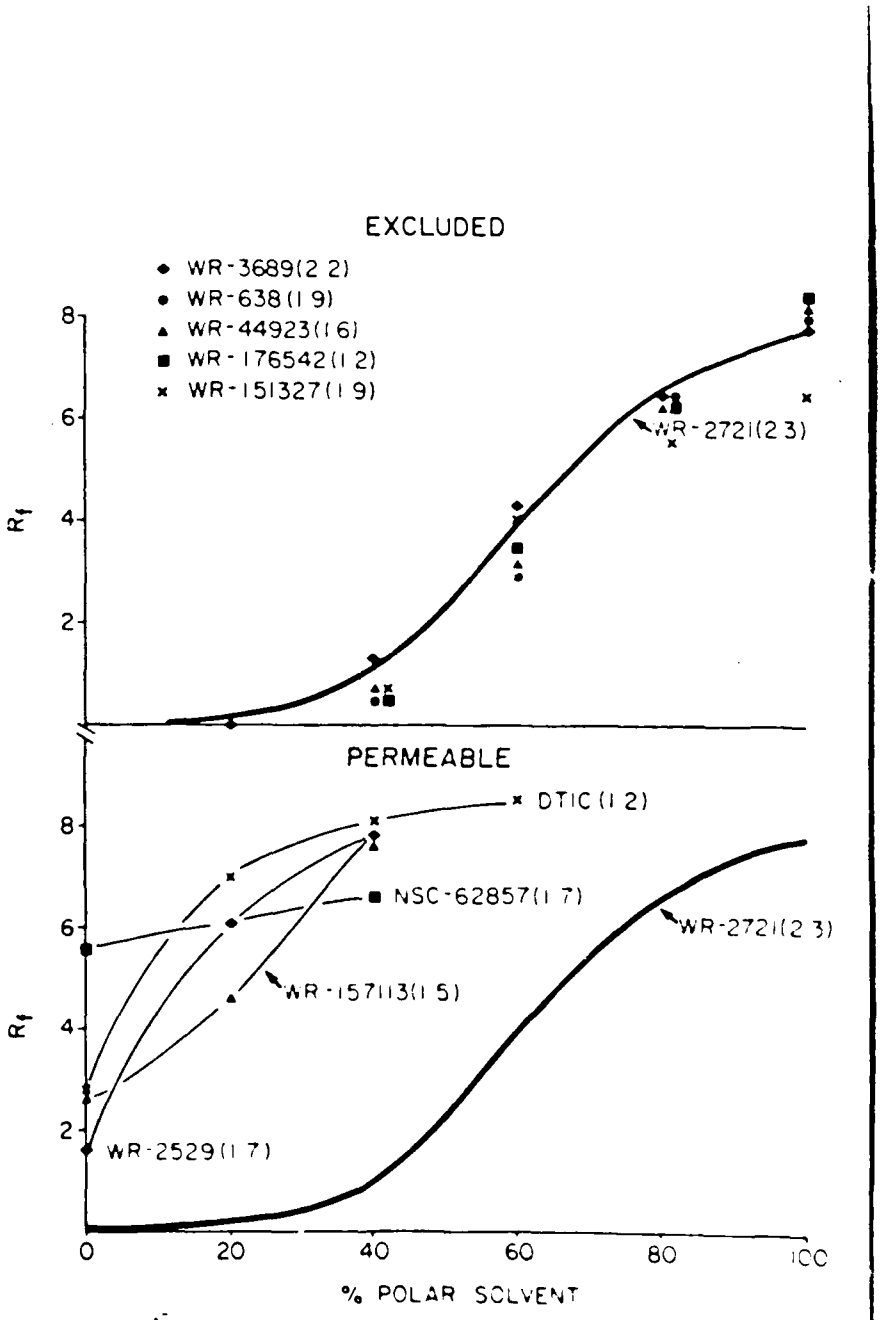


Figure V. Categorization of candidate radioprotective drugs as to whether they would be excluded from the CNS or be permeable, based on their relative migration in an isopropanol:ammonium hydroxide solvent system.



LITERATURE CITED

Sweeney, T.R. (1979). A survey of compounds from the Antiradiation Drug Development Program of the USAMR&DC, Walter Reed Army Inst. of Research, Washington, D.C., 851 pp.

Yuhas, J.M. and Storer, J.B. (1969). Int. J. Radiat. Biol. 15:233-238.

Yuhas, J.M. (1968). Radiat. Res. 35:714-721.

Yuhas, J.M. (1979). Brit. J. Cancer 40:161-163.

Yuhas, J.M. (1980). Cancer Res. 40:1519-1524.

Yuhas, J.M. et al. (1980). In, Radiation Sensitizers, L. Brady, Ed., Masson Publ., USA., pp. 303-308.

END

4-87

DTIC